Bioorganic Chemistry **29,** 234–257 (2001)
doi:10.1006/bioo.2001.1211, available online at http://www.idealibrary.com on IDEAL®

PLP and PMP Radicals: A New Paradigm in Coenzyme B_6 Chemistry

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Received June 19, 2000

Enzymes frequently rely on a broad repertoire of cofactors to perform chemically challenging transformations. The B_6 coenzymes, composed of pyridoxal 5′-phosphate (PLP) and pyridoxamine 5′-phosphate (PMP), are used by many transaminases, racemases, decarboxylases, and enzymes catalyzing α,β and β,γ-eliminations. Despite the variety of reactions catalyzed by B_6-dependent enzymes, the mechanism of almost all such enzymes is based on their ability to stabilize high-energy anionic intermediates in their reaction pathways by the pyridinium moiety of PLP/PMP. However, there are two notable exceptions to this model, which are discussed in this article. The first enzyme, lysine 2,3-aminomutase, is a PLP-dependent enzyme that catalyzes the interconversion of L-lysine to L-β-lysine using a one-electron-based mechanism utilizing a [4Fe-4S] cluster and *S*-adenosylmethionine. The second enzyme, CDP-6-deoxy-L-*threo*-D-*glycero*-4-hexulose-3-dehydrase, is a PMP-dependent enzyme involved in the formation of 3,6-dideoxysugars in bacteria. This enzyme also contains an iron–sulfur cluster and uses a one–electron based mechanism to catalyze removal of a C-3 hydroxy group from a 4-hexulose. In both cases, the participation of free radicals in the reaction pathway has been established, placing these two B_6-dependent enzymes in an exclusive class by themselves.

Key Words: enzyme mechanisms; coenzyme B_6; pyridoxal 5′-phosphate (PLP); pyridoxamine 5′-phosphate (PMP); lysine 2,3-aminomutase; deoxysugars; E_1; E_3; radicals; iron–sulfur proteins.

INTRODUCTION

The power and versatility of enzyme catalysis is a testament to the ability of nature to devise mechanisms in order to navigate the landscape of evolution. Nature has elegantly refined proteins to enhance their capabilities to catalyze a wide variety of reactions which are chemically challenging under physiological conditions. The catalytic mechanisms of a large number of enzymes rely solely on the inherent properties of the amino acid residues in their active sites. However, in addition to the active site residues, many enzymes have to "accessorize" themselves with coenzymes such

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0045-2068/01 $35.00

as organic cofactors or metal ions and clusters to carry out more mechanistically demanding tasks. The incorporation of coenzymes as part of the holoenzyme complex enables the proteins to significantly magnify the breadth and versatility of their catalysis.

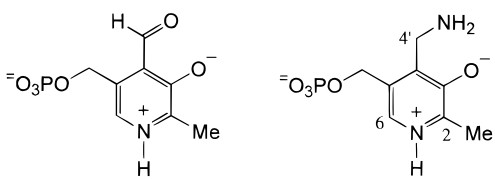
Among the known organic cofactors, the vitamin B_6 coenzyme plays a central role in the metabolism of amino acids and amines. This coenzyme, comprising a pyridine ring which is protonated in the enzyme active site, exists in two different forms, pyridoxal 5′-phosphate (PLP, **1**) and pyridoxamine 5′-phosphate (PMP, **2**) (Fig. 1). The electrophilic nature of the charged pyridinium ring enables the B_6 coenzyme to stabilize α-anionic intermediates and thus facilitate transaminations, racemizations, decarboxylations, α,β eliminations, and β,γ eliminations of amino acids and amines (*1*). Numerous coenzyme B_6-dependent enzymes are known, almost all of which utilize the B_6 coenzyme based on its capability to act as an electron sink, reinforcing their mechanistic paradigm.

As more examples of catalysis involving the PLP- and PMP-dependent enzymes emerged, two interesting cases were discovered in which the enzymes perform their tasks with the assistance of B_6 coenzyme by one-electron chemistry. In this article, the mechanisms of these two enzymes will be discussed in detail. The first enzyme, lysine 2,3-aminomutase, uses PLP to catalyze the reversible conversion of L-lysine to L-β-lysine. The second enzyme, CDP-6-deoxy-L-*threo*-D-*glycero*-4-hexulose-3-dehydrase (E_1), uses PMP for the C-3 deoxygenation of a 4-keto-6-deoxyhexose in the biosynthesis of bacterial 3,6-dideoxysugars. Despite the differences in the reactions catalyzed by these enzymes and their cofactor requirements, the common theme shared by them is that their reaction mechanisms involve the formation of radical intermediates, which is contrary to the established paradigm for PLP/PMP-dependent enzymes. The discovery of these reactions has again served as an indicator of the versatility of enzyme catalysis and as a reminder of the new vistas of nature's chemistry that might still remain unexplored.

LYSINE 2,3-AMINOMUTASE

Introduction

Lysine 2,3-aminomutase (LAM) catalyzes the interconversion of L-lysine (**3**) and L-β-lysine (**4**) (*2*). This enzyme has been found in *Clostridium* and *Streptomyces*. Recently, the isolation of lysine 2,3-aminomutase from *Bacillus subtilis* has also been reported (*3*). In contrast to the enzyme from other sources, lysine 2,3-aminomutase



1 *2*

FIG. 1.

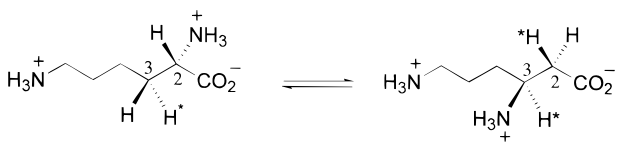
from *B. subtilis* is stable even when purified under aerobic conditions. In *Clostridia*, the formation of L-β-lysine is the first step in the metabolism of lysine to acetyl-CoA and ammonia that are used as the source of the carbon and nitrogen building blocks by the organism (*4*). Conversely, in *Streptomyces*, L-β-lysine is a secondary metabolite which is used for the acylation of amino groups in a variety of antibiotics (*5–8*).

As shown in Scheme 1, the forward reaction catalyzed by lysine 2,3-aminomutase must involve the cleavage of the unactivated C–H bond at C-3 of **3**, with concomitant migration of the amino group from C-2 to C-3 to form **4**. Such a cleavage of an α-C–H bond to bring about an enzyme-catalyzed 1,2 rearrangement of the target functional group is well precedented and is a role typically performed by vitamin B_{12} (adenosylcobalamin)-dependent enzymes (Scheme 2) (*9*). However, what makes the lysine 2,3-aminomutase reaction intriguing is that the activity of this enzyme is not dependent on adenosylcobalamin or other derivatives of vitamin B_{12}, but on an unlikely combination of cofactors as described in the next section.

Physical and Chemical Properties

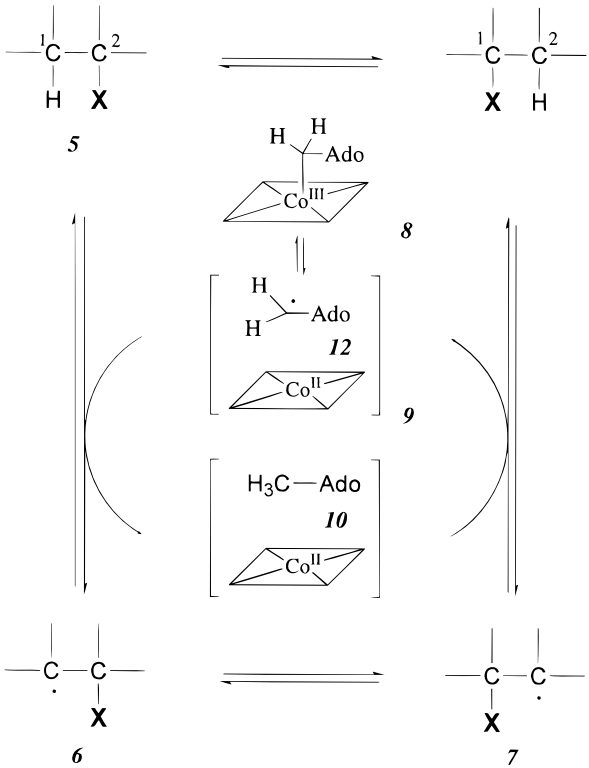
The lysine 2,3-aminomutase from *Clostridium subterminale* SB4, first purified in 1970, is the best studied member of this class of enzymes (*10,11*). It exists as a hexamer in the native form with a molecular mass of approximately 47 kDa per subunit (*12*). The reaction catalyzed by this enzyme is reversible with an equilibrium constant of 7 at pH 8.0 in favor of L-β-lysine (*10,13*). As shown in Scheme 1, using lysine molecules stereospecifically labeled with deuterium at the appropriate positions, it was established that the hydrogen from the 3-pro-*R* position of L-lysine (**3**) is transferred to the 2-pro-*R* position of L-β-lysine (**4**) (*14*). Consequently, the overall migrations of the hydrogen atom and the amino group proceed with inversion of configurations at both C-2 and C-3. Studies of this reaction also revealed that the transfer of the amino group is intramolecular. However, only part of the hydrogen migrating from C-3 to C-2 was retained during catalysis. Since no exchange of hydrogen between the substrate and the solvent was found, the observed loss of the label was more likely an event occurring between the substrate and the enzyme (*15*).

Interestingly, purified lysine 2,3-aminomutase has no bound corrinoid molecules and is not activated by adenosylcobalamin (*16*). Instead, it contains iron and PLP, and can be activated by *S*-adenosylmethionine (SAM, **11**) (*10,12*). As described earlier, PLP generally serves to stabilize anionic intermediates in transaminations, decarboxylations, α,β-eliminations, and β,γ-eliminations of amino acids and amines, whereas SAM, possessing an activated methyl group, is commonly used as a versatile



3 *4*

SCHEME 1.



SCHEME 2.

biological alkylating agent. Thus, considering the well-established functions of both PLP and SAM in enzyme catalysis, their roles in the 1,2-rearrangement catalyzed by lysine 2,3-aminomutase were not immediately apparent. Subsequent studies to unravel the mechanistic relevance of these cofactors have shown that, indeed, they play very different roles in the catalytic mechanism of lysine 2,3-aminomutase.

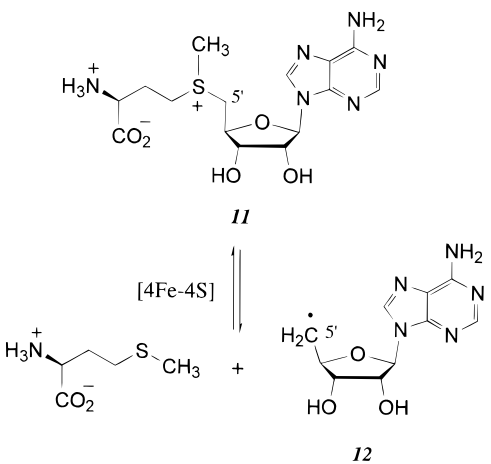
Role of S-Adenosylmethionine

Most enzyme-catalyzed 1,2-rearrangement reactions are adenosylcobalamin-dependent (**8**, Scheme 2) (*17*). The cobalt-carbon bond in this coenzyme, with a bond dissociation energy of ca. 30 kcal/mol, can be readily cleaved homolytically to generate cobalamin(II) and a 5′-deoxyadenosine-5′-yl radical (**9**) (*18,19*). The deoxyadenosyl radical acts as the initiator of catalysis by abstracting a hydrogen atom from the substrate **5** to form a substrate radical **6** and 5′-deoxyadenosine **10**. As illustrated in Scheme 2, the substrate radical **6** then undergoes a rearrangement with the transfer of the migrating group to form the product radical **7**. Finally, the product radical recovers a hydrogen atom from 5′-deoxyadenosine to form the product and regenerate the 5′-deoxyadenosine-5′-yl radical that can be used to initiate another round of catalysis. Reconstitution of the apo-enzymes with [5′-^{3}H]adenosylcobalamin leading

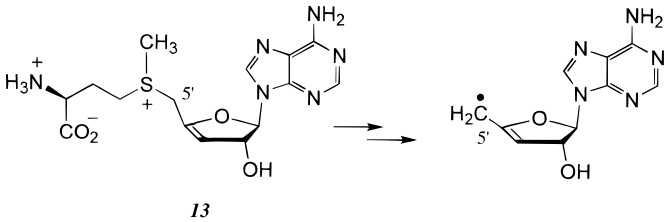
to tritium incorporation in the substrates and products has been commonly used as evidence to substantiate the role of adenosylcobalamin as a mediator of hydrogen transfer in most enzyme-catalyzed 1,2 rearrangements (*20–24*).

In lysine 2,3-aminomutase, however, the corresponding role is carried out by SAM (**11**), which serves as the source of the 5′-deoxyadenosine-5′-yl radical upon cleavage of the bond between the sulfonium group and the deoxyadenosine moiety (Scheme 3). This has been shown in experiments where the activation of lysine 2,3-aminomutase by *S*-[5′-^{3}H]adenosylmethionine led to a similar incorporation of tritium into lysine and β-lysine (*25*). The reverse transfer of tritium from β-[3-^{3}H]lysine into SAM has also been confirmed (*26*), lending further credence to the participation of SAM in hydrogen exchange. The hypothesis that SAM plays a role analogous to that played by adenosylcobalamin is consistent with the fact that hydrogen transfer from C-2 to C-3 in lysine 2,3-aminomutase has been shown to occur in a partially intramolecular fashion. Any of the three 5′-methyl hydrogens of 5′-deoxyadenosine can be transferred to the product radical; thus, the hydrogen derived from a given substrate molecule may or may not be returned back to its product.

Although the formation of a 5′-deoxyadenosine-5′-yl radical has been postulated in the catalytic mechanisms of B_{12}- as well as a few SAM-dependent enzymes (*27–30*), this radical has not been directly observed, presumably due to its instability. However, a recent study on lysine 2,3-aminomutase has provided compelling spectroscopic evidence for the formation of this radical (*31*). The authors incubated lysine 2,3-aminomutase with lysine and a SAM analog, *S*-3′,4′-anhydroadenosyl-L-methionine (**13**) (Fig. 2). The analog was designed with the assumption that the allylic radical formed at the 5′-position would be sufficiently stable to be observed by spectroscopic methods. Indeed, the EPR spectra of samples containing lysine 2,3-aminomutase, lysine, and **13** showed the formation of a carbon-centered radical at $g = 2.002$. Characteristic changes in the spectral patterns obtained by the incubation of isotopically labeled analogs of lysine and **13** with lysine 2,3-aminomutase indicated that



SCHEME 3.



13

FIG. 2.

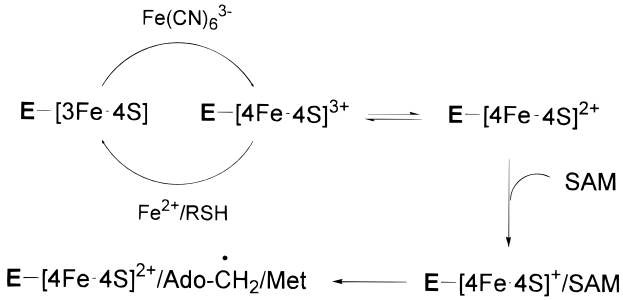
this radical was localized at the C-5′ of **13**. The results from this report provide the first conclusive evidence for the formation of the 5′-deoxyadenosine-5′-yl radical **12** in the mechanism of lysine 2,3-aminomutase and support its formation in other B_{12}/SAM-dependent enzymes.

Role of the Iron–Sulfur Center

The first report on the purification of lysine 2,3-aminomutase indicated the presence of approximately 3 mol of iron per mole of enzyme hexamer (*10*). Subsequent development of a strict anaerobic protocol led to an enzyme containing 10–12 mol of iron per mole of hexamer, with a stoichiometric amount of inorganic sulfide (*16*,*32*). The direct correlation of enzyme activity to the iron and sulfur content, the reduction in activity upon exposure of lysine 2,3-aminomutase to air, and the exhibition of an absorption band at 410 nm in the active protein indicated the presence of a catalytically essential iron–sulfur center in lysine 2,3-aminomutase. Further analysis determined the iron-sulfur center to be a [4Fe–4S] cluster (*33*).

Using EPR spectroscopy, four different states of the putative [4Fe–4S] cluster have been identified (*33*). These include three different oxidation states of the [4Fe–4S] center: [+1], [+2], and [+3], and a [3Fe–4S] cluster derived from the [+3] state. However, the iron–sulfur center of lysine 2,3-aminomutase, when isolated, exists as a mixture of the [+3] and [+2] states. The [+1] state, which can be obtained by treatment of the [+2] state with a strong reducing agent in the presence of SAM, was shown to be catalytically relevant. The requirement for SAM in this process may be related to a conformational change of the enzyme induced by its binding that raises the reduction potential of the [+2] state, allowing the enzyme to be reduced to the [+1] form. Such a hypothesis is supported by the fact that binding of *S*-adenosyl-homocysteine (SAH) also allows the generation of the [+1] form of the iron-sulfur center (*31*). Further experiments have shown that the formation of the [+1] state is practically irreversible and the enzyme can undergo multiple turnovers without reverting to the [+2] state or releasing SAM. The interconversion of the different states of the iron-sulfur cluster of lysine 2,3-aminomutase is shown in Scheme 4.

After the active [+1] form of lysine 2,3-aminomutase is generated, cleavage of SAM to give 5′-deoxyadenosine-5′-yl radical and methionine takes place. This cleavage is reversible and likely occurs through interactions of the iron–sulfur center with SAM in the enzyme active site. Four possible mechanisms have been proposed for this



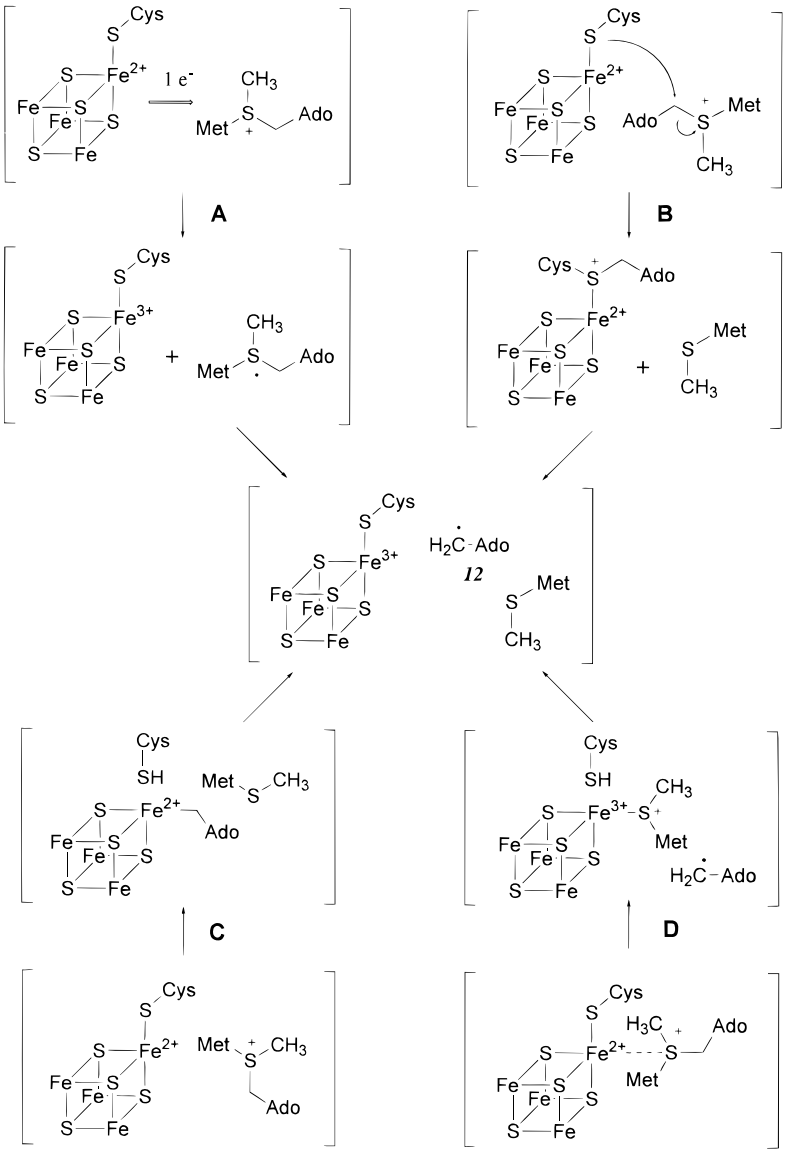
SCHEME 4.

process (*2*,*34*). As depicted in Scheme 5, the iron–sulfur cluster may be oxidized from the [+1] state to the [+2] state by transferring an electron to the sulfur atom in SAM (mechanism A). The reduced form of SAM is expected to be unstable, and could fragment into the 5′-deoxyadenosine-5′-yl radical and methionine. In mechanism B, a sulfur atom of one of the [4Fe–4S] ligands attacks C-5′ of SAM in a nucleophilic fashion, leading to the alkylation of the iron–sulfur cluster and the cleavage of the $C_{5'}$–S bond. The alkylated cluster then dissociates into the 5′-deoxyadenosine-5′-yl radical and the oxidized iron–sulfur center. An analogous alkylation of SAM involving one of the iron atoms from the cluster instead of the sulfur ligand is proposed in mechanism C. This intermediate then breaks down to form the adenosyl radical and the oxidized cluster. In mechanism D, SAM is proposed to have a direct interaction with the iron atom in the cluster through coordination from the sulfonium ion. Electron transfer from the cluster to the sulfonium ion results in cleavage of SAM and the formation of the adenosyl radical and methionine. The formation of methionine results in a tighter coordination of the sulfur atom to the iron cluster, which could provide some of the driving force for electron transfer.

A recent report has provided evidence for the direct interaction of SAM and the [4Fe–4S] cluster during the formation of the 5′-deoxyadenosine-5′-yl radical (*35*). Using selenium K-edge X-ray absorption spectroscopic analysis of LAM in the presence of selenomethionine and Se-adenosyl-L-selenomethionine, it was found that the cofactor was cleaved only when a reducing agent and substrate analog were present. Furthermore, the spectroscopic data indicated that the SAM analog was cleaved to selenomethionine, which remained associated with the iron–sulfur cluster. Based on these observations, it appears that Mechanism D is most likely responsible for the reductive cleavage of SAM to generate the 5′-deoxyadenosine-5′-yl radical.

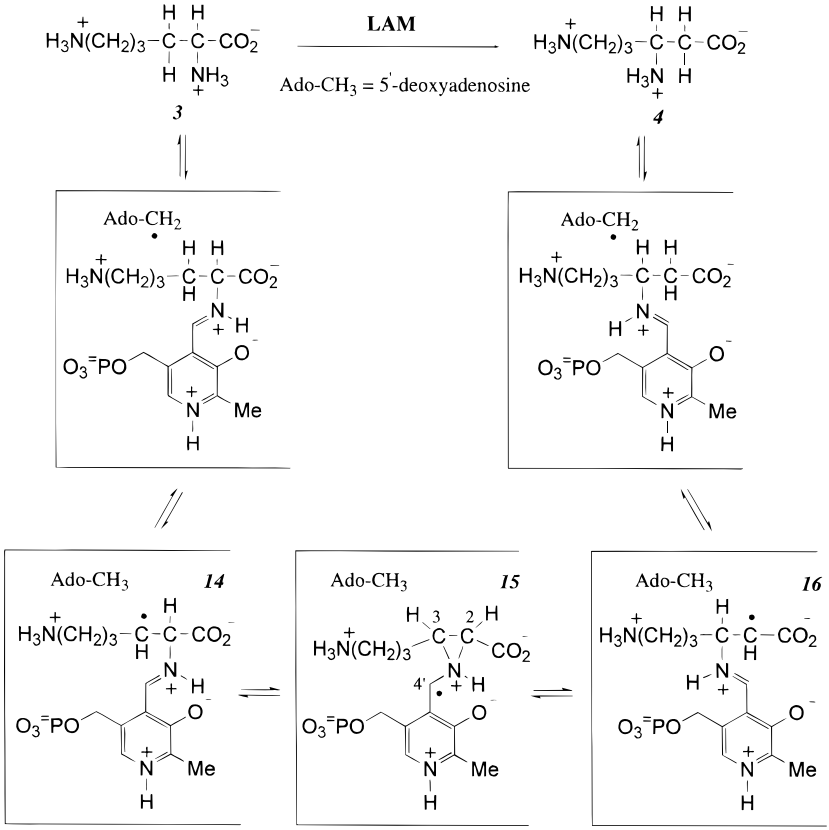
Role of Pyridoxal 5′-Phosphate

While the detailed steps leading to the formation of the 5′-deoxyadenosine-5′-yl radical **12** in lysine 2,3-aminomutase remains shrouded in ambiguity, the subsequent steps involving PLP are better understood (*25*). In the resting state, the PLP cofactor forms a Schiff-base with a lysine residue (Lys-346) (*36*). As illustrated in Scheme 6, the first chemical step after binding the substrate is the formation of an external aldimine between the α-amino group of lysine and the C-4′ aldehyde group of PLP.



SCHEME 5.

The actual catalysis is expected to be initiated by the putative 5′-deoxyadenosine-5′-yl radical that abstracts the C-3 hydrogen of lysine to form 5′-deoxyadenosine and the substrate radical **14**. Rearrangement of **14** to the product radical **16** is facilitated by PLP through the formation of a putative aziridylcarbinyl radical **15** as shown in Scheme 6. The aziridylcarbinyl radical can undergo ring opening by two possible



SCHEME 6.

routes: it can break the bond between the bridging C-4′ nitrogen and either C-3 or C-2 of lysine. The former pathway reverts to the substrate radical, while the latter leads to the formation of the product radical, which can abstract a hydrogen from the 5′-methyl group of 5′-deoxyadenosine to yield the external aldimine of β-lysine. The product is then removed by hydrolysis of the Schiff base, leaving the enzyme ready to initiate another round of catalysis. This mechanism adequately accounts for the unusual requirement of PLP in the lysine 2,3-aminomutase reaction, as well as in reactions of other aminomutases (*18*).

Spectroscopic Observation of Radical Intermediates

When lysine 2,3-aminomutase is mixed with lysine, an EPR spectrum with a complex pattern of couplings centered at $g = 2.001$ is observed at 77 K (*37*). The g value indicates that the signal most likely corresponds to an organic radical. The radical species behaves like an intermediate generated during the interconversion between lysine and β-lysine, since the concentration of the radical is the highest within the first 30 s, after which it decays at a rate matching that of the equilibrium

between lysine and β-lysine (*37*). The EPR signal was broadened when [2-^{13}C]lysine was used as the substrate, a characteristic phenomenon observed with other π-radicals (*38*). Conversely, the spectral features were simplified and narrowed when [2-^{2}H]lysine was used as the substrate, indicating that majority of the splitting observed with the original signal was due to coupling with the C-2 hydrogen. Based on the change in the coupling patterns by isotopic labeling of the substrate, this EPR signal was assigned to the product radical **16** shown in Scheme 6. The fact that only the product radical is observable by EPR spectroscopy may simply be due to the delocalization of an appreciable amount of electron density of the radical by the C-1 carboxyl group leading to increased stability of the radical. Further analysis by resolution enhancement of the EPR spectra obtained with [2-^{2}H]lysine, [U-^{2}H]lysine, and [2-^{2}H, 2-^{15}N]lysine, allowed the determination of all relevant hyperfine splitting constants for the radical. Using this data, the dihedral angle between the π-orbital harboring the unpaired electron spin and the C-3 hydrogen was calculated to be about 70°, and that between the π-orbital and the C-3 nitrogen was estimated to be about 10° (*39*,*40*).

Recently, EPR-spectroscopic data has also been obtained for the lysine analog *trans*-4,5-dehydro-L-lysine (**17**) (*41*) (Fig. 3). This compound was designed with the assumption that if it is recognized as a substrate by LAM, the initial hydrogen abstraction at C-3 should give rise to a radical that is stabilized by the C_4–C_5 double bond. Thus, the resulting allylic radical should be stable enough to accumulate and be detected by EPR experiments. Indeed, incubation of **17** with LAM resulted in the appearance of a strong EPR signal with the characteristics of an organic radical. Several deuterated analogs of *trans*-4,5-dehydro-L-lysine (**18**–**22**) were also synthesized, and the resulting changes in EPR spectral patterns upon the incubation of these analogs indicated that the unpaired electron was located on the carbon skeleton, with the spin density residing on both C-3 and C-5. However, detailed analysis of the hyperfine splitting constants revealed that the spin density was unevenly distributed, with 60% of the density residing at C-3. Kinetic analysis of the EPR signal indicated that the rate of formation of the radical matched that of the decrease of the $[4Fe–4S]^{+}$ EPR signal, implying that the two processes are coupled.

Interestingly, 4-thia-L-lysine (**23**) is also a substrate for lysine 2,3-aminomutase

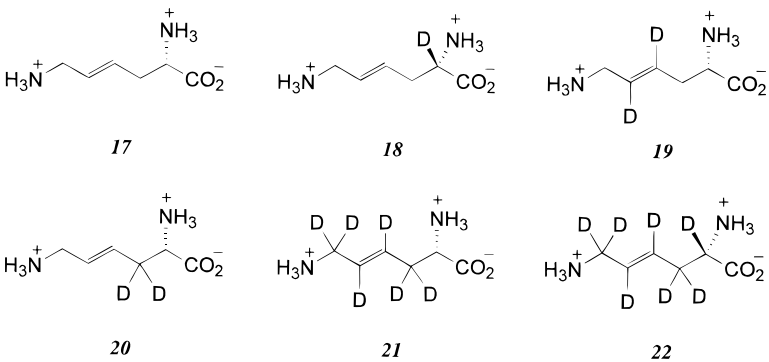


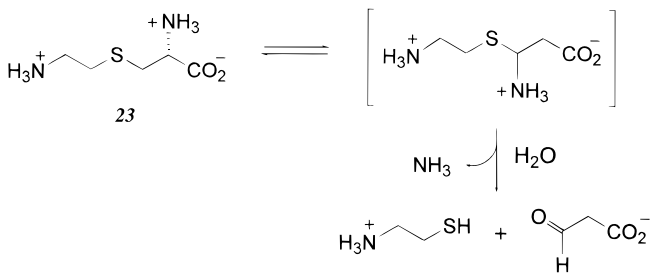
FIG. 3.

(Scheme 7) (*42*). The maximum rate of turnover with **23** is only about 3% of that for the reaction of lysine, even though their K_m values fall in the same range. When the steady-state of the turnover of 4-thia-L-lysine is probed by EPR spectroscopy, a strong EPR signal is observed. This EPR signal also corresponds to an organic radical intermediate as judged by the *g* value of 2.003. However, the spectral shape of this signal is very different from that observed with radical **16**. The spectrum is significantly broadened by the use of 4-thia-L-[3-^{13}C]lysine as a substrate and is narrowed with the use of 4-thia-L-[3-$^{2}H_2$]lysine. Taking these results into consideration, it seems that the unpaired electron spin predominantly exists on C-3 of 4-thia-L-lysine in the steady-state. The C-3 radical (**14**), which is not detected in the reaction of lysine, is presumably stabilized by the presence of the *p*-orbitals on the neighboring sulfur atom in **23**, enabling it to accumulate to sufficiently high concentrations to be detected by EPR spectroscopy.

To determine the influence of PLP on the radicals, the enzyme was reconstituted with [4′-^{2}H]PLP and mixed with SAM and lysine. Analysis of the generated radical species by deuterium ESEEM spectroscopy revealed a strong doublet at the NMR frequency of deuterium. The distance between the radical and C-4′ was estimated to be 3.4 Å based on the coupling constant, supporting the location of PLP near the product radical (*43*).

Summary

Studies on the mechanism of lysine 2,3-aminomutase have revealed that this enzyme uses a variety of cofactors in an unusual manner to bring about a challenging transformation. The role played by SAM as a source of the deoxyadenosyl radical is an interesting departure from its usual function as a biological methylating agent. A few other enzymes where SAM is thought to play a similar role include pyruvate formate-lyase-activating enzyme (PFL-AE) (*27*), anaerobic ribonucleotide reductase (ARNR) (*28*), biotin synthase (*29*), and, most recently, LipA, a protein involved in lipoic acid biosynthesis (*30*). However, in lysine 2,3-aminomutase, the deoxyadenosyl radical directly reacts with the substrate, while in PFL and ARNR it is involved in the generation of the catalytic species, a protein-based glycyl radical. Preliminary evidence has suggested that in biotin synthase, a deoxyadenosyl radical generated by SAM is also directly involved in the reaction pathway (*44*); however, it is consumed in



SCHEME 7.

stoichiometric amounts in this enzyme, whereas it is used catalytically in lysine 2,3-aminomutase.

The role played by the PLP cofactor in lysine 2,3-aminomutase also deviates significantly from its normal role in enzyme catalysis. There are no anionic intermediates involved in the reaction mechanism which might need stabilization. Instead, the reaction occurs through a radical mechanism. It can be argued that the role of PLP in this context might be to provide a degree of stabilization to the aziridinylcarbinyl radical species **15** that is proposed to exist between the substrate and product radicals in the reaction coordinate. In fact, a recent computational study revealed that the introduction of a PLP functionality in conjugation with a 2-aminoethyl radical substantially reduced the energy barrier for the vicinal exchange of the radical and the amino group (*45*). Theoretical calculations indicated that the introduction of an imine or PLP group facilitated the formation of a discrete three-membered cyclic intermediate along the reaction coordinate. However, the physical whereabouts of this radical remain elusive. In the 1,2 rearrangements of halogen atoms to an adjacent radical site, it has been proposed that the bond between the halogen atom and the two adjacent carbon atoms migrates back and forth at the rate of 10^{11} s^{-1} or greater at ambient temperatures (*46*). By analogy, the interconversion of **14** to **16** could involve a gradual weakening of the bond between C-2 and nitrogen with a concomitant bond formation between C-3 and nitrogen in a concerted manner. Thus, a pathway proceeding via an aziridine-like transition state, instead of involving a discrete aziridine intermediate, remains a mechanistic option.

However, the existence of radicals corresponding to the substrate, product, and cofactor in the reaction mechanism of lysine 2,3-aminomutase is a foregone conclusion, based on the elegant studies that have been described earlier. The involvement of radicals in catalysis is a hitherto unprecedented phenomenon in the chemistry of PLP-dependent enzymes, placing lysine 2,3-aminomutase in a niche of its own.

CDP-6-DEOXY-L-THREO-D-GLYCERO-4-HEXULOSE 3-DEHYDRASE (E_1)

Introduction

Carbohydrates have been the focus of growing attention among biological molecules in recent years due to the increased recognition of their vital roles in many physiological processes (*47*). One class of carbohydrates that has recently gained notability for its importance in living systems is deoxysugars (*48,49*). These sugars are formed by the replacement of one or more hydroxyl groups on a common sugar by hydrogen(s) or other non-*O*-linked functional group(s). These modifications bring about a fundamental change in the chemical properties of the resulting deoxysugars (*50*). No longer serving as metabolic vehicles, they are instead diverted for the execution of other roles where structural stability and specific binding affinity are more important. For example, deoxy sugars are found as pathogenic determinants in the cell walls of bacteria, as indispensable structural components in antibiotics, and as the principal component of the backbone of DNA. In each instance deoxysugars are found to be critical in determining the properties, activity, and the stability of their respective molecular structures.